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A new species and a new record of the genus *Entoloma* from China

Jian-Rui Wang & Tolgor Bau*

Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun 130118, P.R. China

* Correspondence to: *Junwusuo@126.com*

Abstract —Two omphalinoid *Entoloma* species were collected from Shandong Province in China. *Entoloma shandongense* is described as new to science, and *E. rusticoides* is recorded for the first time in China. Detailed descriptions and illustrations of the two species are provided. In addition, molecular phylogenetic analysis based on ITS sequences is also presented.

Key words —*Entolomataceae*, new taxon, morphology, taxonomy

Introduction

More than one thousand species of *Entoloma* have been described worldwide (Kirk et al. 2008). Major works on this genus have been published in the past twenty years (Largent 1994, Noordeloos 2004, Noordeloos & Hausknecht 2007, Gates & Noordeloos 2007, Horak 2008, Noordeloos & Gates 2009, 2012). In China, 137 species of *Entoloma* have been recorded, including several new taxa and new records in recent systematic studies (Li et al. 2008, 2009; He et al. 2010, 2011, 2012, 2013).

Recently, two omphalinoid *Entoloma* species were collected in Shandong Province. One with a blue pileus is described as new to science, and the other represents a first record for China. Morphological characters and the results of ITS sequence analyses are provided for both species.

Materials & methods

Materials were collected from Shandong province, north China and were deposited in the Herbarium of Mycology of Ludong University (HMLD). Morphological characters of the two species were described and illustrated using traditional taxonomic methods.

For morphological studies, color notations follow Kornerup & Wanscher (1978). Microscopic characters were examined using water to observe the color of the tissues,

5% KOH solution as a mounting solvent, 1% Congo Red solution as a staining agent, and Melzer's reagent to detect amyloidy. At least 20 basidiospores, basidia, and cystidia per specimen were measured.

The molecular confirmation was derived from ITS sequences. DNA was extracted according to Sun et al. (2009). ITS rDNA was amplified using primers ITS1 and ITS4. Amplification was performed in 25 μL volumes containing 0.5 μL template DNA, 2.5 μL PCR-buffer (10×), 2 μL Mg2+ (25 mmol/L), 0.2 μL Taq enzyme (5 U/μL), 0.5 μL dNTP mix (10 mmol/L), 0.5 μL of each primer and 17.8 μL ddH_2O. The reaction was carried out with initial denaturation (94°C, 3 min), 35 cycles by the following conditions: denaturation (94°C, 50s), annealing (50°C, 30s), extension (72°C, 50s), final extension (72°C, 10min). The PCR amplification products were sequenced by Sangon Co., Shanghai, China. The target gene sequences were checked using Bioedit (Version 7.0.9; Hall 1999). The final BLASTN was done in Genbank.

Entoloma shandongense T. Bau & J.R. Wang, **sp. nov.** Fig. 1

MycoBank MB 802463

Differs from *Entoloma ater* by its blue pileus and its smaller basidiospores.

Type: China, Shandong Province, Dezhou City, Botanical Garden of Dezhou, 2 September 2011, Jian-Rui Wang (**Holotype**, HMLD 1796; GenBank, KC257440).

Etymology: *shandongense* refers to the type locality, Shandong Province.

Basidiomata small, omphalinoid. Pileus 10–18 mm broad, hemispherical, depressed in the center to infundibuliform, not hygrophanous, slightly translucent-striate almost to center, smooth, mostly blue with somewhat purple tinge (22B5–22B4) over the whole surface, slightly deeper (21C5–21C6) or deep dull blue-violet (20C5–20C6) in the center, tomentose. Lamellae decurrent or adnate with short decurrent tooth, 1.5–2 mm broad, distant, pale pinkish beige (7A1–7A2) to pale pinkish red (11A1–11A2); margin entire. Lamellulae present, 2-3 tiers. Context thin, whitish to pale brown. Stipe central to somewhat eccentric, 10–15 mm in length, 1–2 mm in width, cylindrical to subclavate, gradually narrowing towards base, concolorous with lamellae, pale beige pink, light beige to white at base. Odor and taste not distinctive.

Basidiospores 5.6–7.2 × (7.5–)8.3–10(–10.5) μm, Q = 1.3–1.5, with obvious apiculus, 5–8-angled in side view, thick-walled. Basidia 29–36 × 8–10 μm, clavate, 4-spored, hyaline, thick-walled when mature, homogeneous or with granular contents. Pleurocystidia & cheilocystidia absent. Lamellar trama regular, made up of cylindrical, narrow elements 30–70 × 8–12 μm. Pileipellis a trichoderm of cylindrical hyphae, often narrower than lamellar hyphae, with terminal elements 30–70 × 5–8 μm, pigment intracellular, plasmatic, dark blue with somewhat purple tinge, sometimes forming clots, which dissolve in 5% KOH. Subpellis composed of subhyaline or very pale blue plasmatic pigment. Pileitrama made up of cylindrical elements, hyaline, thin-walled. Stipitipellis composed of hyphae 5–8 μm in diameter, hyaline,

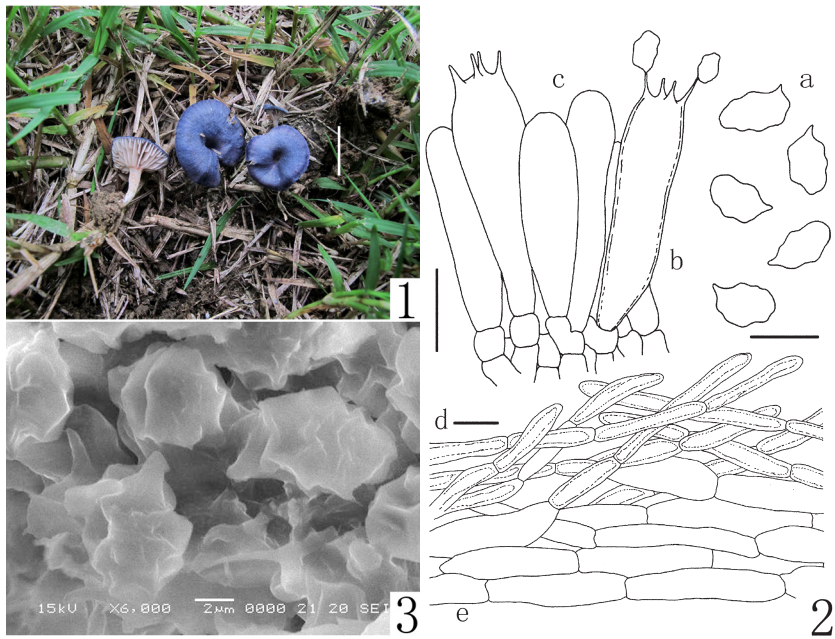

Fig. 1. *Entoloma shandongense* (holotype): 1. Basidiomata (in situ).
2. Line drawings: a. Basidiospores; b. Basidia; c. Young basidia; d. Pileipellis; e. Pileitrama.
3. SEM: Basidiospores. Scale bars: 1 = 10 mm; 2 = 10 μm.

thin-walled, without intracellular pigment. Stipe trama regular, made up of longitudinally cylindrical hyphae up to 5–10 μm in diameter. Caulocystidia absent. Oleiferous hyphae scattered in lamellar trama and pileal trama. Brilliant granules abundant in all tissues. Clamp connections absent.

Ecology — on soil in *Poa pratensis* grassland.

Commentary — The main characteristics of *Entoloma shandongense* are the omphalinoid blue pileus, the many-angled to nodulose spores, and the absence of cystidia and clamp connections.

There are many species with a blue pileus in *Entoloma*, but they are rarely omphalinoid. Only *E. ater* (Hongo) Hongo & Izawa (Hongo 1958) resembles *E. shandongense* in its small omphalinoid basidiomata and grassland association, but the pileus of *E. ater* is often purplish and its basidiospores are larger. Some *Entoloma* species with blue caps lacking the violet hues have recently been described from China (Li et al. 2009, He et al. 2012), e.g., *E. azureosquamulosum* T.H. Li & Xiao Lan He, *E. subaltissimum* T.H. Li & Chuan H. Li, and *E. dinghuense* T.H. Li & Chuan H. Li. *Entoloma azureosquamulosum*

differs from *E. shandongense* in its convex or conical cap and possession of hymenial cheilocystidia and caulocystidia on the stipe. The spores of *Entoloma subaltissimum* are isodiametric and those of *E. dinghuense* are quadrate, neither heterodiametric as in *E. shandongense*.

Other eastern Asian species with blue-violet pilei (e.g., *E. cyanonigrum* (Hongo) Hongo, *E. violaceum* Murrill, *E. glutiniceps* (Hongo) Noordel. & Co-David, *E. kujuense* (Hongo) Hongo) all differ in larger size (pilei often >2 cm broad) and a dark purplish (not blue) coloration. In addition, *E. cyanonigrum* spores are larger (10.4–11.7 × 6.5–9.1um; Katumoto 2010) and *E. glutiniceps* spores are isodiametric (7–8 μm diam.; Co-David 2009), distinguished from the smaller ellipsoid spores of *E. shandongense*. Finally, pleurocystidia and cheilocystidia, absent in *E. shandongense,* are present in both *E. kujuense* (Hongo 1985) and *E. violaceum* (Murrill 1917).

Entoloma rusticoides (Gillet) Noordel., Persoonia 11(2): 150, 1981. Fig. 2

Basidiomata small, mycenoid or omphalinoid. Pileus 8–14 mm broad, convex to hemispherical, sometimes with a small papilla, becoming depressed to umbilicate in the center, hygrophanous, translucent-striate almost to center, smooth, dark gray to umber brown, light beige brown when dry, finely tomentose, center always whitish fibrillose-scaly, margin incurved and undulating. Lamellae pale beige to pale brown-pink, decurrent; margin entire. Context thin, cream-colored to whitish. Stipe central, 10–15 mm in length, 1–1.5 mm in width, cylindrical to subclavate, surface smooth, gray to brown, whitely tomentose at base. Odor faintly herbaceous, Taste mild.

Basidiospores (8.2–)8.5–10.5(–11.0) × (7.6–)8.0–10.3(–11.7) μm, ellipsoid to sub-isodiametric, 5–6-angled in side view, thick-walled, straw coloured. Basidia 28–41 × 9.5–10.5(–12) μm, cylindrical to clavate, 2 or 4-spored, hyaline, thick-walled when mature, clampless. Pleurocystidia & Cheilocystidia absent. Lamellar trama regular, made up of cylindrical elements, 50–100 × 5–15 μm. Pileipellis made up of cylindrical elements 8–20 μm in diameter, septa without clamps, occasional hyphal ends slightly inflated, up to 30 μm in diameter, thin-walled, without clamp connections, externally encrusting pigments brown. Stipitipellis composed of clampless hyphae 8–15 μm, thin-walled, encrusting pigments brown. Stipe trama regular, made up of longitudinal cylindrical hyphae, up to 5–15 μm in diameter, without clamp connections. Caulocystidia absent.

Ecology — in soil alongside paths or associated with *Poa pratensis* or *Cynodon dactylon* grasslands.

Specimens examined — **China**, **Shandong province**, Heze: Zhaowanghe Garden, 7 August 2011, Jian-rui Wang (HMLD 1442; GenBank, KC257438); Liaocheng: Dongchanghe, 1 September 2011, Jian-rui Wang (HMLD 1490; GenBank, KC257441).

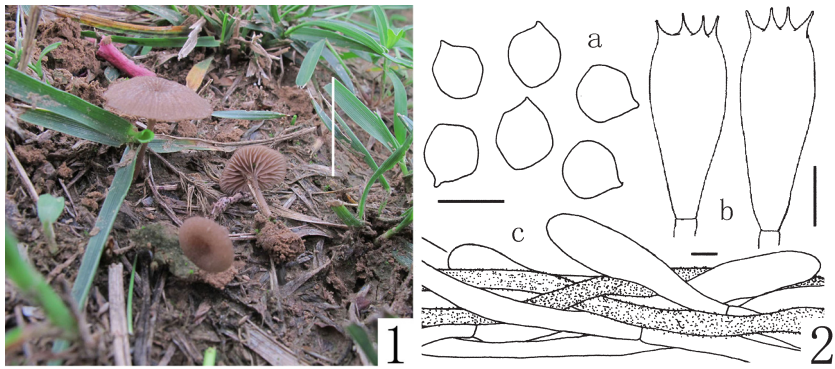

Fig. 2. *Entoloma rusticoides* (HMLD1442): 1. Basidiomata (in situ).
2. Line drawings: a. Basidiospores; b. Basidia; c. Pileipellis.
Scale bars: 1 = 10 mm; 2 = 10 µm.

Commentary — *Entoloma rusticoides* is similar to *E. rhodocylix* (Lasch) M.M. Moser, *E. phaeocyathus* Noordel., and *E. flocculosum* (Bres.) Pacioni in its omphalinoid shape and sub-isodiametric spores. However, *E. rhodocylix* (Moser 1978) and *E. phaeocyathus* (Noordeloos 1985) both have cheilocystidia in the lamellar edge, and *E. flocculosum* (Pacioni 1987) differs in its non-striate, densely floccose-scaly pileus.

Discussion

The morphology-based taxonomic differences of *Entoloma shandongense, E. rusticoides,* and related species have been confirmed by ITS sequence analyses.

The ITS sequence of the *E. shandongense* holotype is similar to those of *E. platyphylloides* (Romagn.) Largent (GenBank JF908003) and *E. undatum* (Gillet) M.M. Moser (GenBank JF908007), with higher query coverage values (≤99%) and max identity (>90%) than other GenBank sequences. However, the three species obviously differ in fruitbody and spore color, shape, and size. *Entoloma platyphylloides,* which occurs in Europe, has a brownish pileus >2 cm in diameter (Kühner & Romagnesi 1954), while *E. undatum* has a gray-white pileus and sometimes possesses cheilocystidia (Moser 1978).

Two ITS sequences from the two *E. rusticoides* specimens are close to those of *E. infundibuliforme* Hesler (GenBank HQ179671), *E. sericeoides* (J.E. Lange) Noordel. (GenBank JF908006), and *E. defibulatum* Arnolds & Noordel. (GenBank JX454928), with higher query coverage values (≤98%) and max identity (≤93%) than other GenBank sequences. However, the spores of *E. defibulatum* (rounded-angular; Arnolds & Noordeloos 1979) and

E. sericeoides (Noordeloos 1980) are smaller than in *E. rusticoides. Entoloma infundibuliformе* has a squamulose pileus and brown cheilocystidia (Hesler 1967) not found in *E. rusticoides.*

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